

THE BACTERICIDAL ACTION OF PROPYLENE GLYCOL VAPOR ON MICROORGANISMS SUSPENDED IN AIR

II. THE INFLUENCE OF VARIOUS FACTORS ON THE ACTIVITY OF THE VAPOR*

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In previous communications (1-2), the killing of air-suspended bacteria by means of very small concentrations of vapors of various compounds, particularly propylene glycol and triethylene glycol, was reported. Under the experimental conditions employed, numerous kinds of bacteria, including pneumococci, hemolytic streptococci, staphylococci, *H. influenzae*, etc., as well as influenza virus, when sprayed into atmospheres containing such vapors, were killed so rapidly that no microorganisms or virus could be recovered from the test chamber. Propylene and triethylene glycols were chosen for especial study, since these compounds are relatively non-toxic and in vapor form are odorless, tasteless, and non-irritating to the respiratory mucosa. The degree of germicidal activity of the vapors was observed to depend upon the concentration of the glycol in the atmosphere. Furthermore, the effectiveness of any given concentration of glycol vapor could be changed markedly by altering certain experimental conditions, such as the volume of culture inoculum atomized into the air, the number of bacteria per unit of volume, the relative humidity and temperature of the atmosphere, and the state of bacterial suspension. The present paper deals with a study of these and other factors which affect or limit the action of propylene glycol vapor. As will be shown in a forthcoming publication the activity of other glycols is similarly influenced.

Methods

The general procedure for carrying out these experiments has been described in the first paper (1). However, since the earlier publication, certain new methods have been developed which have either replaced those employed formerly or are used as supplementary devices.

1. *Colorimetric Method for Quantitative Determination of Glycol Vapors in Air.*—A new method (3) for the determination of glycol vapor in air was adopted because of

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its greater simplicity and speed. The glycol vapor is absorbed in water as in the previous method and the resulting solution analyzed colorimetrically after quantitative oxidation with acidified potassium dichromate. This method permits taking air samples of several cubic feet which makes for increased accuracy when studying the glycol content of large spaces. The color comparison can be performed either with a photoelectric colorimeter or by visual matching of the unknown with a series of standard solutions.

2. *New Apparatus for Collecting Bacteria from the Air.*—A highly efficient means of bacterial air-sampling has been described by Moulton, Puck, and Lemon (4) by which large samples of air can be secured in a relatively short space of time.¹ The apparatus made of glass, utilizes the principle of atomization to collect air-suspended bacterial particles. This is accomplished by drawing air at the rate of 1 cubic foot per minute² through an atomizer containing nutrient broth, aliquots of which are then seeded into blood agar plates. Samples of bacteria-containing air in the experimental room described below could be secured satisfactorily by drawing air through a glass tube inserted through the wall of the room. Such samples yielded just as high bacterial counts as when the apparatus was placed inside the room.

3. *Experimental Room.*—In order to reproduce more closely actual room conditions, experiments were carried out in an experimental room as well as in the 60 liter glass chambers. This room is 10 feet square and 8 feet high, made of plywood and painted on the inside with a shiny enamel of the type used on the walls of hospital wards. Two sides of the room are fitted with glass windows two feet square, a third side has a wooden window, and the fourth a door, with a glass window 2 × 3 feet. In the center of the room on the floor is a fan whose blade is directed at the ceiling, so that a continuous and uniform circulation of air is obtained as shown in Fig. 1. This fan³ operated by a 110 volt motor, was run on 20 volts by means of a Varitran, so as to maintain only a very gentle air circulation. Bacterial suspensions were sprayed into the room by means of a Graeser atomizer (6) mounted in the center of the room above the circulating fan. The apparatus for glycol evaporation was placed in the same general position. The dispersal of dust-borne bacteria is discussed in the section dealing with desiccated microorganisms.

The bacterial content of the air was measured at first by means of the modified Hollaender-DallaValle collector described in the first paper, using 1 and 2 cubic foot air samples. Later settling plates were employed for this purpose. Agar or blood agar plates were placed on the floor near the wall so as to be in the path of the downward air current set up by the fan. The petri dishes could be reached by inserting one's arm through a cloth sleeve attached to an opening in a small door in the wall near the floor. The opening of the sleeve was kept sealed when not in use by a strip of elastic and a metal clip. The plates were exposed for 10 or 20 minute periods during

¹ A simple manometer arrangement devised by Lemon and Wise (5) is especially convenient for measuring these air volumes.

² Air pumps which are very satisfactory for this purpose are made by the Welch Mfg. Co., Chicago.

³ We wish to express our thanks to the Reynolds Electric Co., Chicago, Illinois, for the use of this fan.

the course of 1 or more hours for each experiment. Duplicate control tests were always run. In many experiments air samples of 2 to 3 cubic feet were also obtained with the Moulton air sampler for comparison with the settling plates.

4. *Vaporization of Glycol.*—Several methods were employed for dispersing propylene glycol vapor into the air of the room. One apparatus consisted of a blower which forced a stream of air (up to 130 cubic feet per minute) over a container of glycol heated to 70–80°C., the exact temperature depending on the concentration of glycol vapor desired. While an atmosphere saturated with the glycol vapor could be attained in 45 minutes to 1 hour by this method it was found difficult to produce any desired concentration below saturation. Thus, while satisfactory for studying glycol-

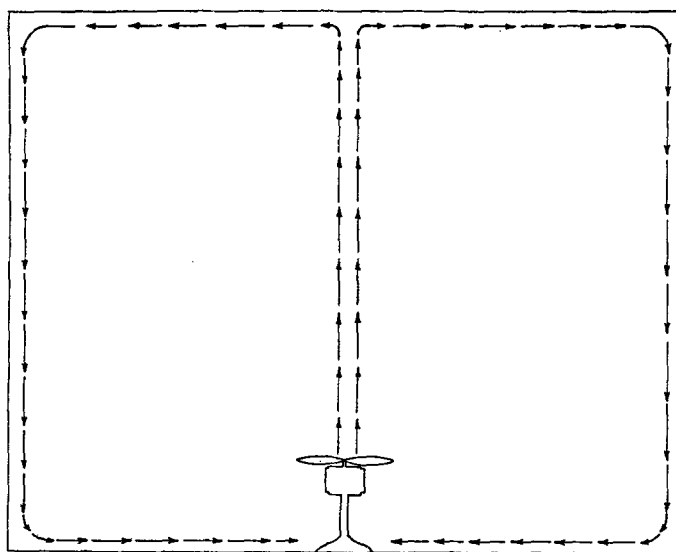


FIG. 1. Schematic diagram of air current produced by fan in experimental room.

saturated atmospheres the apparatus is not suitable for observations on unsaturated ones since the exact concentration of glycol in the air cannot be controlled. A second and more satisfactory method is that of rapidly vaporizing by heat a quantity of propylene glycol which will give the desired concentration of vapor in the air. For this purpose two cylindrical 100 ohm 100 watt, vitreous-enameled, fixed resistor units 6 inches long were connected in series and mounted parallel to one another, almost in contact. The surface of each was covered by very fine monel metal mesh to provide uniform heating and a strand of glass cloth was packed between the two resistors for their entire length, to act as a wick. The unit was placed with one resistor above the other and glycol dropped from a burette onto the upper one. The resistors were connected directly to 110 volt current which provided a moderate amount of heat capable of vaporizing as much as 1 cc. of glycol per minute without any boiling or apparent decomposition. Excess glycol draining off the upper resistor spread over the surface of the lower one and completely vaporized without leakage.

5. *Control of Humidity and Temperature.*—The humidity in the experimental room was raised to the desired level by means of a Walton humidifier⁴ or by means of a steam jet. Experiments at low humidity were performed during the winter when the indoor atmosphere was dry. Humidity determinations were made with wet and dry bulb thermometers. No special means for controlling the temperature of the experimental room was available.

Wide variations in humidity could be secured in the small chambers by flushing them with air dried or moistened to any desired humidity. Temperature regulation was maintained by controlling the surrounding air temperature.

EXPERIMENTAL

Studies on the physicochemical properties of propylene glycol vapor, subsequent to those reported in the first paper, showed that the saturation values given in the literature⁵ are incorrect. In an analysis of the maximum quantities of propylene glycol which can be held in the air at different temperatures and humidities it was found that at 20°C. the vapor pressure is 0.08 mm. instead of 0.18 mm., the published value. Thus at 20°C. and 0 per cent relative humidity the air is saturated with 0.33 mg./liter instead of 0.73 as formerly considered. This value increases with the temperature and decreases with rising relative humidity. For example, at 25°C. saturation occurs at 0.5 mg./liter when the relative humidity is 0 per cent, but only about 0.25 mg./liter can exist in the vapor state when the humidity is 50 per cent. The introduction of amounts of propylene glycol greater than saturation causes condensation of the excess glycol on the walls of the experimental chambers and other surfaces. The condensed glycol re-evaporates whenever the concentration of vapor in the chamber air is lowered below the saturation point. When small chambers are used this condition results in the maintenance of a completely, or nearly completely, saturated atmosphere as long as the glycol evaporation occurs. Since there is good evidence, which will be presented later, that glycol is used up in the process of bacterial killing in the air, knowledge of the saturation values at different temperatures and humidities was found to be essential to the interpretation of experimental results presented below. These data will be published in detail shortly.

Relationship between Numbers of Bacteria Suspended in Air and Glycol Concentrations

A series of experiments was performed to determine the effects of differing concentrations of propylene glycol vapor on varying numbers of *Staphylococcus albus*. The volume of the bacteria-containing fluid which was sprayed into the small glass chambers was kept approximately the same in all experiments. The temperature and humidity were maintained between the levels of 27–30°C. and 44 to 52 per cent relative humidity respectively. The averaged results of twenty experiments are shown in Table I. It is seen that when amounts of propylene

⁴ Walton Laboratories, Inc., 1186 Grove Street, Irvington, New Jersey.

⁵ Synthetic organic chemicals, New York, Carbide and Carbon Chemicals Corporation, 11th edition, 1942, 14.

glycol considerably greater than those required for saturation of the air are introduced (0.66 mg./ liter), there results a pronounced immediate bactericidal action on very large numbers of staphylococci. In three of four tests in which many thousands of microorganisms were recovered in a plate taken from the control chamber immediately (15 seconds) after the introduction of the bacteria, similar plates taken from the test chamber yielded less than one half

TABLE I

Relationship between Numbers of Bacteria Suspended in Air and the Effectiveness of Different Glycol Concentrations Employing Staphylococcus albus as the Test Microorganisms

(Total number of droplets of culture inoculum kept constant. Temperature 27–30°C. Relative humidity about 50 per cent)

Amount of propylene glycol introduced: mg. glycol per liter of air	Calculated glycol concentration in chamber air	No. of bacteria on "immediate" control plate	Per cent reduction in No. of bacteria in glycol chamber* relative to control		
			Immediately (15 sec. after bacterial spray)	5 min. later	15 min. later
0.66	Greater than saturation	4,000–13,000	per cent 99.3	per cent 100	per cent 100
0.4–0.45	Saturation (about) 1:2,500,000	500–6,000	96.7	99.8	100
0.32	Slightly below saturation 1:3,000,000	400–1,400	83.6	99.8	99.6
		6,000	84.4	86	100
0.25–0.27	Unsaturated	73–199	72.7	99.0	97.8
		450–1,300	11.6	36.2	85.7
0.16	1:6,000,000	36–122	0	0	0

* The percentages are based on comparison with the numbers of bacteria in samples taken simultaneously from control chambers; *i.e.*, Per cent reduction =

$$(\text{No. of bacteria from control chamber} - \text{No. of bacteria from glycol chamber}) \div \text{No. of bacteria from control chamber}.$$

of 1 per cent of this number. When the amount of glycol was reduced to about that required for saturation of the air immediate killing of large numbers of microorganisms was pronounced but not quite as marked as when larger amounts of glycol were employed. However, after 5 minutes the effects were the same. As the concentrations of propylene glycol were diminished below the saturation point the rapidity of bactericidal action lessened progressively. These tests with partially saturated atmospheres brought out clearly the relationship of numbers of air-suspended bacteria to bactericidal activity of the glycol; *i.e.*, the more marked effect on the smaller bacterial inocula. Con-

centrations of 0.16 mg./liter or 1:6,000,000 were found to exert no killing effect even on a very few staphylococci.

Relationship between Number of Droplets in Bacterial Spray and Glycol Concentration

In the foregoing experiments a type of atomizer was employed for introducing the bacterial mist which delivered 130 to 150 mg. of fluid in 30 seconds. Calculation of the number of droplets thus dispersed into the air of the chamber gives a figure of approximately 35 billion (250 billion in 1 cc. of culture fluid) assuming that the individual droplets average 2 microns in diameter.⁶ Since the number of bacteria contained in 140 mg. of the inoculum is very much less than the number of droplets resulting from atomization of the culture fluid into the test chamber, it follows that many droplets contain no bacteria. Thus when an inoculum containing one billion bacteria per cc. (the concentration of the standard suspension regularly employed in the tests) is introduced into the chamber in mist form, on the average only one droplet out of 250 would contain a bacterium. In many experiments the standard suspension was diluted 1:10 or 1:100 so that in such instances the ratio of sterile droplets to bacteria-containing droplets was very large. If the explanation of the glycol vapor-bacterial droplet interaction given in the preceding paper is correct, namely that a high concentration of propylene glycol is built up in the bacterial droplets by means of contact with and absorption of the glycol molecules into these droplets, then reducing the volume of the bacterial spray and hence diminishing the total number of droplets should result in a relatively more effective action of the vapor.

To test this inference we employed an atomizer constructed with a finer air orifice which delivered 8 to 12 mg. of fluid in 5 to 20 seconds. The bacterial suspensions were concentrated ten to twenty times so as to yield numbers of bacteria equivalent to those introduced into the test chambers with the large volume atomizers. Such a bacterial mist contained a much higher ratio of bacteria-carrying to non-bacteria-carrying droplets. Pneumococcus Type I was employed as the test microorganism since it is much more sensitive to the bactericidal effect of the glycol vapor than is the *Staphylococcus albus* and hence should be more suitable for observation over a wide range of vapor concentration.

It was found that the bactericidal effect of a given concentration of propylene glycol vapor on approximately the same number of pneumococci was influenced to a considerable extent by the volume of the bacterial suspension atomized into the vapor-containing atmosphere. Table II shows the comparative effects of concentrations of vapor ranging from 0.2 mg./liter to 0.05 mg./liter on com-

⁶ Measurement of the droplets in the Millikan oil-drop apparatus indicated that this was the approximate average size.

parable numbers of pneumococci dispersed from 130 to 150 mg. of suspending fluid on the one hand and 8 to 12 mg. on the other. Each line in the table represents the averaged results of several experiments. With every concentration employed the bactericidal action was more pronounced with the smaller volume of inoculum. While the constitution of the mists, *i.e.* the size of droplets, produced by these two types of atomizers may not be exactly the same, our observations on this point suggest that the difference in droplet size is probably slight. Hence, it seems that the number of fluid droplets in the air has an important influence on the bactericidal activity of the glycol vapor.

TABLE II
Relationship between Number of Droplets in Bacterial Spray and Glycol Concentration.
Pneumococcus Type I

(Total number of bacteria dispersed into air kept fairly constant.)

Relative volume of bacterial suspensions sprayed into chamber	Calculated concentration of propylene glycol vapor	No. of colonies on immediate (15 sec.) control plate	Per cent reduction in No. of bacteria in glycol chamber relative to control		
			15 sec.	5 min.	15 min.
			<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
Large*	0.20	213	70	96.5	99.5
Small*	0.20	211	100	100	100
Large	0.15	195	68	90.5	97.5
Small	0.15	424	80	100	100
Large	0.10	153	16	76.5	88
Small	0.10	577	53	94	100
Large	0.05	485	24	9	45
Small	0.05	203	70	82	90

* Ratio of volume of large inoculum to small was approximately 14:1.

Effect of Temperature

A study of the effect of temperature changes on the bactericidal action of glycol vapors was undertaken in order both to gain an insight into the mechanism of this process and to determine how changes of temperature such as ordinarily occur in inhabited rooms might affect the practical use of these vapors. For these studies, the two 60 liter chambers were placed in a thermostatically regulated, constant-temperature room. The room temperature was set to the desired value and the chambers allowed to reach thermal equilibrium with the room before the start of each experiment.

The effect of temperature changes on the bactericidal action of propylene glycol vapor is shown in Table III. The conditions of the experiments were so arranged that the concentration of the glycol employed (1 gm. in 5,000,000 cc. of

air) permitted observation of the rate of killing of *Streptococcus hemolyticus* Group C over a period of 15 minutes. The size of the bacterial inoculum was such as to produce approximately 400 colonies on a plate taken immediately after the bacterial spray. The relative humidity was kept at 50 per cent.

The results presented in the table are the averaged values obtained for a series of three or more experiments at each temperature. The per cent reduction at 15 minutes for each chamber, glycol and control respectively, was calculated as follows:

$$\frac{\text{Immediate plate count} - 15 \text{ minute plate count}}{\text{Immediate plate count}} \times 100 = \text{per cent reduction at 15 minutes}$$

In order to obtain the net per cent reduction, the value for the control chamber (which represents the natural rate of disappearance of bacteria from the air)

TABLE III

Bactericidal Action of Propylene Glycol Vapor at Various Temperatures with Relative Humidity of 50 per cent. Streptococcus hemolyticus Group C. Glycol Concentration 0.20 Mg./Liter

Temperature inside chamber	Per cent reduction in No. of bacteria in 15 min.		Net reduction due to glycol
	Test chamber	Control chamber	
°C.	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
37	78.5	50.2	28.3
25	86.1	42.8	43.3
15	100	39.4	>60.6*

* Since all the test plates were sterile by, or before, 15 minutes the exact per cent kill at 15 minutes cannot be known. The true figure must be greater than 60.6, however.

was subtracted from that of the glycol chamber. This figure shown in the last column of the table represents the bactericidal action of the glycol.⁷

The data of Table III show a marked increase in bactericidal efficiency at lower temperatures. Such a negative temperature coefficient indicates that the limiting step in the chain of events leading to the destruction of air-borne bacteria by propylene glycol vapor is a physical process rather than a chemical reaction.

Effect of Humidity

That the relative humidity of the atmosphere could exert a very profound effect on the bactericidal action of glycol vapors was to be expected because this factor determines the degree of hydration of the air-suspended bacterial

⁷ This method of representing the data was chosen for the sake of simplicity. The results can be expressed with greater mathematical accuracy in terms of the slope of the graph of the logarithm of the number of bacteria plotted against the time. Both methods yield essentially the same result.

droplets. Such droplets will evaporate, perhaps even to dryness when the humidity is very low, but will remain moist at higher humidities. Experiments⁸ have shown that the rate of evaporation of such droplets in dry atmospheres is extremely rapid; droplets freshly sprayed into the air may evaporate to a small fraction of their original diameter within a few seconds. Furthermore, at high relative humidities, the concentration of glycol molecules which can exist in the gaseous state is limited. Propylene glycol and water possess a very high affinity for each other, and can unite in any proportion to form a solution. The number of glycol molecules capable of existence in the vapor state is therefore

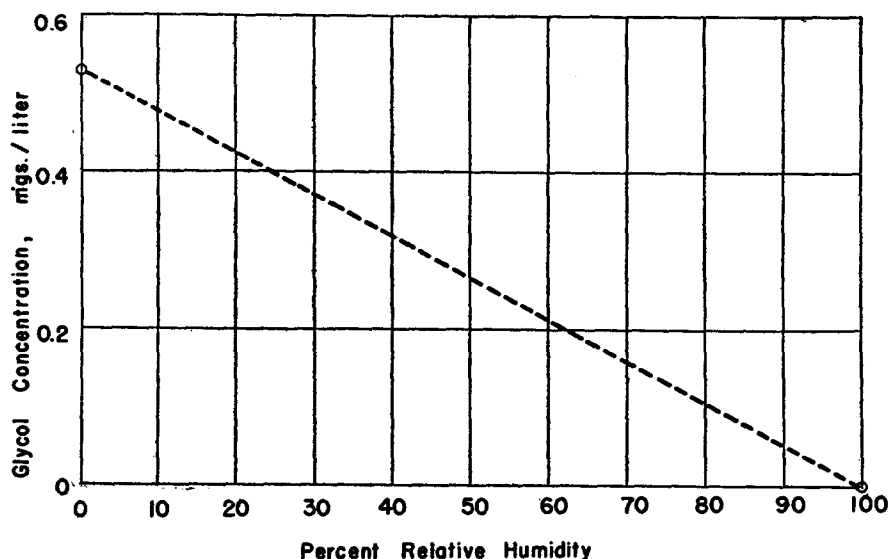


FIG. 2. Maximum glycol concentration capable of existence in vapor state. Theoretical curve calculated on basis of Raoult's law. Temperature = 25°C.

decreased when water vapor is also present because of the tendency of these two kinds of molecules to condense out together as a liquid. Thus, as the humidity of the air increases, the maximum amount of glycol vapor which can coexist without supersaturation, must decrease. These relationships are shown in Fig. 2. Only the first and last points on this curve are experimental. The straight line was drawn assuming Raoult's law to hold. The actual curve is now being determined in this laboratory. The indications are that the deviations from the theoretical straight line are not greater than 15 per cent.

The effect of the relative humidity on the killing of air-suspended *Streptococcus hemolyticus* Group C by propylene glycol vapor was studied at a temperature of 77°F. The experimental conditions and procedure were the same

⁸ These measurements were made by observing droplets in the Millikan apparatus. They will be presented in a forthcoming paper.

as those described in the series dealing with the effect of temperature at a constant humidity.

The results of these experiments at humidities ranging from 27 per cent to 91 per cent are shown in Fig. 3. Each point on the curve represents the average of a number of experiments. The method of representing the data is the same as that used in Table III. The curve reveals that at both very low and very high humidities the bactericidal efficacy is markedly reduced. The optimum range lies between relative humidities of 45 per cent and 70 per cent with a peak at about 58 per cent.⁹

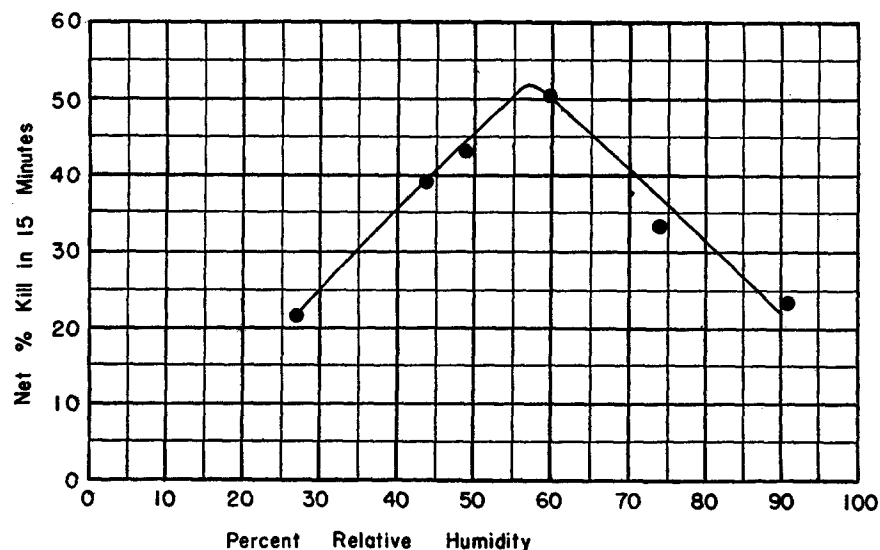


FIG. 3. Killing action of propylene glycol vapor at various relative humidities. Temperature = 77°F.

Because of the great practical importance associated with conditions of low relative humidity such as occur indoors in the wintertime, when the spread of infections is usually high, the killing action of glycol vapor in dry atmospheres was given special study. The diminution in effectiveness of these vapors at low humidities was observed with all the microorganisms tested. A typical experiment with *Pneumococcus* Type I is presented in Table IV. The data indicate that under identical conditions of glycol concentration and temperature, and employing approximately the same numbers of microorganisms only a

⁹ Baker and Twort (7) found that the germicidal activity of their various aerosols was influenced by humidity and appeared to be most effective in the range of 40 to 60 per cent. Challinor (8) also reported the efficacy of hypochlorite sprays to be increased at high humidities.

slight reduction in numbers was obtained with a relative humidity of 15 per cent, whereas a marked bactericidal effect occurred at 42 per cent relative humidity.

Even at extremely low humidities, however, propylene glycol vapor was found to be strongly and rapidly bactericidal if present in a sufficiently high concentration. This action was demonstrated by filling a chamber with air which had been thoroughly dried by passage through a long CaCl_2 tube. In addition a large pan of drierite was placed in the chamber, covering almost the entire floor. A humidostat inside the chamber registered less than 13 per cent relative humidity. Propylene glycol vapor was introduced in an amount to yield a concentration of 0.60 mg./liter. The temperature inside the chamber was 31°C. Under these conditions, complete and immediate sterilization of the air occurred when even a very large inoculation of *Staphylococcus albus* was sprayed

TABLE IV
Effect of Low Humidity on Bactericidal Action of Propylene Glycol Vapor
Pneumococci Type I Sprayed into Chamber Containing Glycol in Concentration of 0.20
Mg./Liter

Relative humidity <i>per cent</i>		No. of colonies on plates taken		
		Immediately (15 sec.)	At 5 min.	At 15 min.
15	Test	186	138	80
	Control	264	234	226
42	Test	254	3	0
	Control	612	388	286

into the chamber. A control chamber, treated in an identical manner but without glycol, yielded plates with hundreds of colonies. It should be emphasized that in this experiment the microorganisms were introduced in the form of a spray of fluid droplets. In the section dealing with dust-borne organisms, it will be shown that similar effects have not been obtained with organisms which are naturally suspended in dust from occupied rooms.

Tests in Experimental Room

The experimental room was used for several types of investigations which for various reasons were difficult or impossible to carry out in the small glass chambers, and for the purpose of determining whether glycol vapors acted as effective air-sterilizing agents in relatively large enclosed spaces. Experiments were first performed in which the air was saturated or supersaturated with glycol vapor at concentrations of 0.4 mg./liter or more which produced a visible fog or condensation on the walls or windows. The vapor was introduced with the blower device described earlier and determinations of the glycol content of

the air were made before introduction of the bacteria. *Staphylococcus albus* suspensions atomized into such atmospheres were killed within a few seconds or minutes after the termination of the bacterial spray. Settling plates opened immediately following the spray were frequently sterile. One experiment is shown in Table V.

Analyses of the air made immediately after the introduction of the glycol vapor into the room failed to give as high a concentration of propylene glycol as would be expected from the amount vaporized by the electric heater. This loss which varied from 30 to 50 per cent of the calculated concentration may be attributed principally to condensation of the glycol on the walls and windows of the room and to leaks to the outside.¹⁰ The difference between the calculated or expected glycol concentration and the concentrations found by analysis of the air was a fairly constant one under identical conditions of humidity, temperature, and movement of the air by the cen-

TABLE V
Effect of Propylene Glycol Vapor on Staphylococcus albus in Experimental Room of 800 C. Ft. Capacity

Plates opened at	Duration of plate exposure	No. of colonies on plates in	
		Control room	Room filled with glycol vapor in concentration of 1/2,500,00 (0.4 mg./liter)
	<i>min.</i>		
Beginning of bacterial spray	30	2,556	11
End of spray	25	2,080	0
20 min. after spray	3½	252	0

trally placed fan. For example the vaporization of 4 cc. of propylene glycol into the room at 28°C. and 50 per cent relative humidity yielded an analysis of 0.1 mg./liter (1:10,000,000) while the calculated concentration was 0.18 mg./liter or about 1:5,500,000.

Studies on the bactericidal action of only partially saturated atmospheres in the experimental room showed that the vapor was fully as effective as in the small glass chambers.

Effect of Kind of Medium in Which Bacteria Were Suspended

Comparative studies were made on bacteria suspended in broth, 10 and 20 per cent rabbit serum-broth, saliva, and water. In the case of *Staphylococcus albus*, propylene glycol vapor appeared to be equally bactericidal for micro-

¹⁰ The room was sufficiently tight however, that following the spraying of bacteria such as Group C hemolytic streptococci into the room, none of these microorganisms could be recovered in 10 cubic foot samples of air just outside the room.

organisms suspended in any one of these fluids. Tests on beta hemolytic streptococcus group C, *Streptococcus viridans*, and the pneumococcus suspended either in broth or saliva gave essentially the same results. It was found, however, that fluids containing little or no protein were unsatisfactory for the suspension of such bacteria as the pneumococcus and hemolytic streptococci, since these fluids fail to protect the microorganisms against mechanical trauma during the process of atomization. Saliva was chosen as the most suitable medium because the dispersal of bacteria in droplets of saliva represents the state in which air-borne bacteria of respiratory tract origin are initially distributed into the environment under natural conditions.

Effects of Degree of Desiccation of Bacteria and Bacterial Droplets

Since fluid droplets such as we have employed in the foregoing experiments when dispersed into air of ordinary humidity evaporate very rapidly and ultimately settle in the dust of the room from which they may again be distributed into the atmosphere by air currents, it became essential to determine the effectiveness of glycol vapor on dried and partially dried bacteria.

The action of propylene glycol on partially desiccated bacterial droplets such as one might expect to be present in the neighborhood of coughing or sneezing patients with active respiratory disease, was tested as follows. Several kinds of bacteria, pneumococcus, hemolytic streptococcus Group A and hemolytic streptococcus Group C were suspended in fresh unsterile saliva and sprayed into the experimental room. In each instance a period of time ranging from 35 to 40 minutes was allowed to elapse before introduction of the glycol vapor. Settling plates were made at frequent intervals during this time and in some experiments 2 to 3 cubic foot samples of the room air were taken with the Moulton apparatus. Gentle agitation of the air of the room was maintained in order to simulate natural conditions. It was found that immediately after vaporization of the glycol, a sharp decrease in number or complete disappearance of these microorganisms which had been suspended in the air for 35 to 40 minutes occurred. One experiment in which the glycol was introduced 40 minutes following the bacterial spray is shown in Table VI. These experiments also revealed the fact that all the various forms of bacteria present in normal saliva were killed by the glycol.

Observations on completely desiccated bacteria were first carried out on *Staphylococcus albus* dried in the frozen state. The bacterial powder was thoroughly ground with dry sterile room dust in a mortar in order to achieve more effective dispersal of the bacteria into the air. The bacteria-dust mixture was placed in a 500 cc. Erlenmeyer flask from which it was blown into the room by means of an air stream under a pressure of about 300 mm. Hg introduced through the top of the flask. The arrangement used is shown in Fig. 4. To achieve a more uniform dispersal of the dust, the procedure was later modified by screening the bacteria-laden dust through a screen

of 120 mesh per inch and mixing the fine powder so produced with crystalline alundum, to prevent re-coalescence of the particles. The flask was shaken gently during the passage of the air stream so that all the surfaces became exposed to the current. The air stream could easily be adjusted so that all the fine dust was blown out of the flask, leaving clean white alundum remaining.

TABLE VI

Action of Propylene Glycol Vapor on Pneumococcus Type I Suspended in Unsterile Saliva

Time after spray of microorganisms <i>min.</i>	No. bacteria collected on a 10 min. blood agar settling plate	Bacteria per c. ft. of air recovered by Moulton air sampler
0-10	107	
10-20	71	88
20-30	40	
30-40	28	110
41-49	4.4 cc. propylene glycol vaporized into room to give a concentration of 0.2 mg./liter	
49-59	0	
59-69	0	0
69-79	0	
79-89	0	0

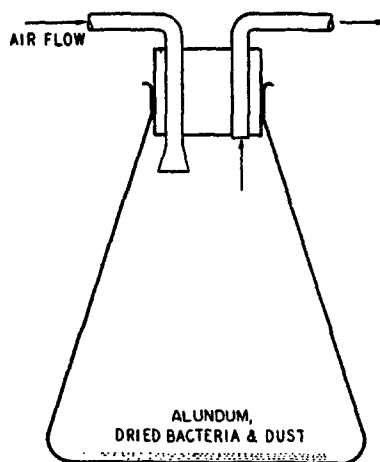


FIG. 4. Apparatus for distributing dried bacteria and dust into atmosphere.

An illustrative experiment is shown in Table VII. In this experiment the control was performed first, following which the room was thoroughly cleared, the vapor then introduced, and the same quantity of bacteria blown into the room as was used in the control. While the bactericidal effect of the glycol vapor was not quite as rapid as when the staphylococci were introduced in fluid droplets nevertheless an immediate pronounced killing occurred and by the end

of 8 minutes the atmosphere was essentially sterile. Analogous effects with similar concentrations of propylene glycol vapor were obtained with dried and dust-mixed *Streptococcus viridans* and hemolytic streptococcus Group C.

Preliminary observations indicate that higher humidities are required for the optimum bactericidal effects with dried bacteria than are necessary when bacteria are in fluid suspension.

Effect of Propylene Glycol Vapor on Unsterile Dust from Occupied Rooms

In order to approximate more closely conditions which may obtain in an enclosed, inhabited space, unsterile dust was collected from various rooms and blown into atmospheres containing propylene glycol vapors. Control experiments without propylene glycol were also carried out. Although occasionally a

TABLE VII
Effect of Propylene Glycol Vapor on Dried and Dust-Suspended Staphylococcus albus in Experimental Room

	Plates opened at	Duration of plate exposure	No. of colonies on plates from	
			Control room	Room filled with glycol vapor in concentration of 1/3,000,000
Bacterial dust sprayed into chamber for 3½ min.	Beginning of spray	min. 20	820	120
	End of spray	20	796	62
	8 min. later	20	644	1
	15 min. later	20	380	2
	20 min. later	20	204	0

marked reduction in the number of bacteria recoverable seemed to be obtained, usually very little effect was noted from the glycol vapors. Whether this failure of action of the vapor is due to a very high degree of resistance of these microorganisms which have survived in room dust for fairly long periods or to the protective action which certain kinds of dust may afford to bacteria, or to failure of such particles to become properly hydrated when dispersed in the air, remains to be determined. These problems are receiving study.

Minimum Glycol Concentration for Effective Bactericidal Action

As the quantity of glycol vapor in the air is decreased, the time required for bactericidal action increases. This is illustrated in the experiment shown in Table VIII wherein three different concentrations of glycol were tested on approximately the same numbers of *Staphylococcus albus* under identical conditions.

Although atmospheres into which propylene glycol had been introduced in quantities in excess of saturation were immediately and completely bactericidal for every microorganism tested,¹¹ partially saturated atmospheres exhibited

TABLE VIII

Relationship between Glycol Concentration and Rate of Bactericidal Action on Staphylococcus albus

Concentration of propylene glycol introduced into chamber	Per cent reduction in No. of bacteria in glycol chamber relative to control		
	15 sec. after bacterial spray	5 min. later	15 min. later
mg./liter	per cent	per cent	per cent
0.55	100	100	100
0.45	92	100	100
0.25	0	46	92

TABLE IX

Comparative Susceptibility of Various Microorganisms to Atmospheres Containing Propylene Glycol Vapor in Quantities below the Saturation Level

Temperature 21–29°C. Relative humidity about 50 per cent.

Propylene glycol vapor concentration	Kind of microorganisms	Per cent reduction in No. of bacteria in glycol chamber relative to control	
		At 5 min.	At 15 min.
mg./liter		per cent	per cent
0.21–0.30	Pneumococcus Type I	98.5	100
	<i>Staphylococcus albus</i>	79.6	93.5
	Hemolytic streptococcus Group A	63	93
	<i>Streptococcus viridans</i>	52	63
0.11–0.20	Pneumococcus Type I	85.7	96.5
	Hemolytic streptococcus Group A	56.5	86
	Hemolytic streptococcus Group C	45.9	66.1
	<i>Staphylococcus albus</i>	11	26
0.05–0.10	Pneumococcus Type I	31.5	54.4
	Hemolytic streptococcus Group A	20	35.1
	<i>Streptococcus viridans</i>	10	21

considerable variation in the rate at which they produced killing of different bacteria. The averaged results from a number of experiments employing

¹¹ These include *Staphylococcus albus*, *Streptococcus hemolyticus* Groups A and C, *Streptococcus viridans*, Pneumococcus Types I and III, *Staphylococcus aureus*, *B. subtilis*, *B. coli*, *Micrococcus catarrhalis*, *H. influenzae*, *H. pertussis*, and several unidentified small Gram-positive cocci isolated from the air.

several microorganisms and a range of glycol concentrations from 0.05 to 0.30 mg./liter are presented in Table IX.¹²

These data indicate that the pneumococcus is the most susceptible of the various organisms tested. Indeed 30 minutes' exposure to a concentration of 0.05 mg./liter of propylene glycol vapor was frequently found to effect a reduction of 95 per cent or more in the number of air-borne pneumococci. Concentrations as small as 0.02 mg./liter sometimes produced complete killing in 30 to 60 minutes. Probably the most resistant of these four microorganisms is the *Staphylococcus albus*, since no evidence of a bactericidal effect has been obtained with glycol concentrations of 0.16 mg./liter or lower within the period of an hour.

A further demonstration of this selective action was obtained by dispersing propylene glycol in a concentration of 0.10 mg./liter into an atmosphere containing both pneumococci and hemolytic streptococci Group C in fine droplet form. The pneumococci were killed very rapidly but relatively little effect on the rate of disappearance of the streptococci was observed.

DISCUSSION

The influence exerted by the various factors here described on the bactericidal efficiency of propylene glycol vapor supports the picture of the mechanism proposed in the previous communication of this series (1). The effects produced by each of these factors can be explained in terms of the extent to which they cause an increase or decrease of condensation of glycol vapor on the air-suspended particles. For example, lethal action was found to be greatest when the number of air-suspended droplets was very small (Table II). Under these circumstances the amount of glycol available for condensation on each droplet is much greater than when a large number of droplets is present.

The progressive reduction in bactericidal action with rising temperature (Table III) can be similarly understood. As the temperature rises the vapor pressure of the glycol (*i.e.* its tendency to evaporate) increases so that there

¹² The accuracy of these figures probably is not greater than 15 to 20 per cent because the glycol concentrations in individual experiments were not uniformly spread over the concentration ranges indicated in the table and because of unavoidable variations in experimental conditions which may even include changes in sensitivity of bacteria to the glycol action. An instance of such an apparent change in the microorganism was observed during the course of repeated determinations of the minimal effective glycol concentration on a Group A hemolytic streptococcus. During the several months following isolation from the patient this streptococcus was killed by a concentration of propylene glycol as low as 1 gm. in 50 million cc. of air. Subsequently, however, similar results could not be obtained with concentrations less than 1:10 million.

results a progressive decrease in the amount of glycol accumulating in the droplets. That the increase in temperature *per se* does not depress the bactericidal activity of propylene glycol has been shown by experiments *in vitro*. Changes in temperature between 15° and 37°C. were found to produce only slight changes in this action of the glycol and in the direction of increasing effectiveness with rising temperature.

These same principles may be applied to explain the rôle of the atmospheric relative humidity. The decreased effectiveness of propylene glycol vapor at low humidities is probably due to desiccation of the bacteria-containing droplets. Experiments have shown that such droplets sprayed into a dry atmosphere evaporate with great rapidity (a matter of 1 or 2 seconds) to a very small size. If these droplets were to lose their surface moisture for which the glycol has a high affinity, they would present to the surrounding atmosphere a dry outer crust offering little attraction for condensation of glycol molecules. Hence the bactericidal action would be less pronounced. At high relative humidities, a somewhat different situation obtains. Although extensive glycol condensation occurs, so much water vapor is present in the atmosphere that it too condenses in the droplet. Thus, a steady state is reached wherein the ratio of glycol to water within each droplet cannot exceed a certain fixed amount, which is determined by the atmospheric humidity. The higher the humidity the smaller is this ratio, *i.e.* the lower the percentage of glycol in the droplet, so that the bactericidal action is diminished or even lost.

In an effort to elucidate further the mechanism of the killing action of propylene glycol vapor, tests on the bactericidal activity of varying concentrations of this substance for the several microorganisms employed in the study, were carried out *in vitro*. These experiments will be presented more fully in a paper dealing with the mechanism of the bactericidal process. Suffice it to state here that in general analogous differences in bacterial susceptibility to propylene glycol were found to hold *in vitro* as were observed in the air. Thus, the pneumococcus which had exhibited the greatest sensitivity to glycol vapor was also found to be the most susceptible to the lethal action of propylene glycol *in vitro*, while the *Staphylococcus albus*, relatively resistant to vapor action, was correspondingly affected to a much less degree in the test tube.

Tests *in vitro* also threw light on the effect illustrated in the fourth line of Table I, namely, that a given concentration of glycol vapor is more rapidly bactericidal against a relatively small inoculum of microorganisms, even though the total number of droplets in the air is kept constant. Propylene glycol was found to exhibit the characteristic behavior of many bactericidal agents *in vitro* in that the smaller the number of microorganisms in the test suspension the more rapid the lethal effect.

In a consideration of the practical utilization of glycol vapors for the destruction of air-borne bacteria, the rôle played by dust presents a problem of great

importance. The lack of any demonstrable bactericidal effect of propylene glycol on microorganisms contained in room dust suggests that certain types of union between dust and bacteria can protect the latter against the action of the vapor, even at favorable humidities. The nature of the protection afforded by dust is not clear. It may involve the action of repellent surface films which resist penetration by either glycol or water, and so prevent contact between these agents and the microorganisms; or, if the dust particles are sufficiently large it would be impossible to obtain within a reasonably short time sufficient condensation of glycol molecules on such particles to produce a bactericidal concentration. In order to effect sterilization of ordinary room air it is clearly necessary to acquire much more information concerning the physical state of microorganisms associated with dust and to develop means of effective dust control.

SUMMARY

A study of the conditions which affect the bactericidal action of propylene glycol vapor on air-suspended microorganisms has been carried out. The killing process was found to be more effective when both the total number of air-borne droplets and the number of organisms in the bacterial suspension are small. A temperature below 80°F. and an atmospheric relative humidity between 45 and 70 per cent were found to constitute the most favorable conditions for the lethal action of the vapor.

Experiments were performed to test the bactericidal efficiency of propylene glycol vapor in both small and large enclosed spaces. These studies revealed that equally marked bactericidal action is obtained when propylene glycol is dispersed in an 800 cubic foot room as occurs in chambers of 2 cubic foot capacity. The susceptibility to vapor action of bacteria re-suspended in saliva was just as great as when broth was used as the suspending medium. Both partially and completely dehydrated bacteria also succumbed to the effects of the vapor. However, when unsterile dust collected from inhabited rooms was dispersed into the air, little reduction of the natural microbic population contained in this material was observed.

Data are presented showing the minimum glycol concentration necessary for effective bactericidal action on various microorganisms. Pneumococci were killed by amounts of propylene glycol as low as 1 gm. in 20 million cc. of air. Concentrations of 1 to 5 million to 1 to 10 million were required to produce the same degree of killing of streptococci and staphylococci.

The observations here reported add further support to the previously proposed conception of the mechanism of the lethal action of propylene glycol vapor, namely, that a bactericidal concentration of the glycol accumulates in the bacterial droplet as a result of contact with and absorption of glycol molecules from the surrounding atmosphere.

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